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**WO 97/07136 (27.02.1997 Gazette 1997/10)****(54) PHARMACEUTICAL COMPOSITION CONTAINING INHIBITORS OF INTERFERON-GAMMA**PHARMAZEUTISCHE ZUSAMMENSETZUNG ENTHALTEND INTERFERON-GAMMA  
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COMPOSITION PHARMACEUTIQUE CONTENANT DES INHIBITEURS DE L'INTERFERON GAMMA

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EP 0 845 007 B1

## Description

[0001] The present invention concerns pharmaceutical preparations for promoting the healing of wounds or fibrotic disorders, in particular for promoting the healing of wounds or fibrotic disorders with reduced scarring, and for promoting the healing of chronic wounds.

[0002] By "wounds or fibrotic disorders" is meant any condition which may result in the formation of scar tissue. In particular, this includes the healing of skin wounds, the repair of tendon damage, the healing of crush injuries, the healing of central nervous system (CNS) injuries, conditions which result in the formation of scar tissue in the CNS, scar tissue formation resulting from strokes, and tissue adhesion, for example, as a result of injury or surgery (this may apply to e.g. tendon healing and abdominal strictures and adhesions). Examples of fibrotic disorders include pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, and proliferative vitreoretinopathy.

[0003] In particular, there is a lack of compositions for promoting the healing of wounds or fibrotic disorders with reduced scarring. Scar tissue formation, although providing mechanical strength to a healed wound, can be unsightly and may impair the function of the tissue.

[0004] This is particularly the case in wounds which result in scar tissue formation in the CNS, the scar tissue inhibiting the reconnection of severed or re-growing nerve ends, so significantly affecting their function.

[0005] There is also a lack of compositions for use in the treatment of chronic wounds, for example venous ulcers, diabetic ulcers and bed sores (decubitus ulcers), especially in the elderly and wheel chair bound patients. Such compositions may be extremely useful in patients where wound healing is either slow or in whom the wound healing process has not yet started. Such compositions may be used to "kick-start" wound healing and may then be used in combination with compositions (e.g. those of PCT/GB93/00586) which promote the healing of wounds or fibrotic disorders with reduced scarring. Hence not only may a chronic wound be healed, but it may be healed with reduced scarring.

[0006] According to the present invention there is provided an inhibitor of IFN- $\gamma$  (Interferon- $\gamma$ ) for use in promoting the healing of wounds and fibrotic disorders with reduced scarring.

[0007] IFN- $\gamma$  (Type II or immune interferon) is produced primarily by T lymphocytes upon mitogen or antigen stimulation (Trinchieri *et al.*, 1985, Immunology Today, 6: 131). IFN- $\gamma$  (both murine and human) exert their effects through specific, saturable, binding to a single class of high affinity receptors found on a variety of cells including fibroblasts, endothelial cells and monocytes/macrophages.

[0008] IFN- $\gamma$  has been widely studied (see, for example, Kovacs, E.J., 1991, Immunology Today, 12(1): 17-23 - who states that IFN- $\gamma$  decreases fibroblast proliferation and connective tissue production, i.e. inhibits

scar tissue formation). Past studies of the effects of IFN- $\gamma$  at wound sites have shown (Pittel, B. *et al.*, 1994, Plastic and Reconstructive Surgery, 93: 1224-1235) that in studies on the effect of intralesional injection of IFN- $\gamma$  to hypertrophic scars (an abnormal thickening of muscle), most (6/7) patients showed relief of symptoms, and all patients showed reduced lesion size during treatment, although there was no change in the total collagen content. Duncan *et al* (1985, J. Exp. Med., 162: 516-527) and Amento *et al* (1985, J. Clin. Invest., 76: 836-848) have shown that IFN- $\gamma$  inhibits collagen types I and III and fibronectin synthesis by dermal and synovial fibroblasts and collagen type II by chondrocytes in a dose-dependent manner. Murray *et al* (1985, J. Immunol., 134: 1619-1622) have also shown that IFN- $\gamma$  is involved in macrophage activation *in vivo*. Tamai *et al* (1995, J. Invest. Dermatol., 104: 384-390) have shown that IFN- $\gamma$  is involved in the regulation of metalloproteases (MMP) and tissue inhibitor of metalloproteases (TIMP) in *in vitro* cell culture.

[0009] It appears that IFN- $\gamma$  is a multi-potent molecule with many actions depending on the conditions of the environment to which it is added. Several groups have reported decreased collagen synthesis *in vitro* on addition of IFN- $\gamma$  to cultures, and Granstein *et al* (1989, J. Invest. Dermatol., 93: 18-27) have shown inhibition of collagen deposition and hence healing with reduced scarring in wounds treated with IFN- $\gamma$ . From these results, it appears that the treatment of sites (of wounds or fibrotic disorders) with IFN- $\gamma$  would result in healing with reduced scarring.

[0010] Experiments undertaken (see 'Experimental' section below) have shown that, very surprisingly, the inhibition of IFN- $\gamma$  actually promotes healing with reduced scarring, despite the teachings of the prior art.

[0011] The inhibitor may, for example, be a neutralising antibody. It may be a monoclonal antibody, a polyclonal antibody, a phage-derived antibody, a genetically engineered antibody (e.g. diabody), or antibody derived from a transgenic mouse.

[0012] Alternatively, the inhibitor may be anything which inhibits IFN- $\gamma$  from interacting with its receptor (i.e. antagonises IFN- $\gamma$  receptor activation) or which inhibits the receptor's activation. It may, for example, be a molecule which mimics the IFN- $\gamma$  receptor binding sequence and which binds to the receptor but does not activate it, thereby competitively inhibiting the binding of IFN- $\gamma$  to the receptor and inhibiting the activation of the receptor.

[0013] The inhibitor may be used in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

[0014] The inhibitor may be used in conjunction with a composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

[0015] The inhibitor may be used in conjunction with a composition for promoting the healing of chronic wounds.

[0016] Also provided according to the present invention is a method for promoting the healing of wounds or fibrotic disorders with reduced scarring comprising inhibiting IFN- $\gamma$ .

[0017] The inhibition may be achieved by administering to a site an inhibitor of IFN- $\gamma$ . By "site" is meant a site of wounding or fibrotic disorder. The inhibitor may be an inhibitor according to the present invention.

[0018] Between about 300 and about 30,000 IU IFN- $\gamma$  may be inhibited.

[0019] The IFN- $\gamma$  may be inhibited immediately prior to wounding/onset (by "onset" is meant the onset of a fibrotic disorder). It may be inhibited immediately after wounding/onset, although it may also be inhibited later, for example within approximately 3 or 7 days of wounding/onset.

[0020] The method may be used in conjunction with a method for promoting the healing of wounds or fibrotic disorders with reduced scarring.

[0021] The method may be used in conjunction with a method for promoting the healing of chronic wounds.

[0022] The invention will be further apparent from the following example which shows, by way of example only, forms of inhibition of IFN- $\gamma$  and promotion of healing with reduced scarring, and of promotion of healing of chronic wounds.

## EXPERIMENTAL

### Method

[0023] 84 male CD1 mice, 12 to 15 weeks old (Charles River) were anaesthetised using equal parts halothane, oxygen and nitrous oxide. 2 x 1cm full-thickness incisions (through the panniculus carnosus) were made 3cm from the base of the skull and 1cm either side of the dorsal midline.

[0024] Test solutions used were anti-IFN- $\gamma$ , IFN- $\gamma$  and PBS. Anti-IFN- $\gamma$  comprised monoclonal antibody against murine IFN- $\gamma$  (MuIFN- $\gamma$ ; = rat IgG'2a). Antibodies were obtained as ascites fluid from thymusless nude mice inoculated with the F3 hybridoma clone (J. Immunol., 1987, 138: 4178) and purified by affinity chromatography on an anti-rat kappa-chain mAb. The neutralisation potential of the antibody was 1/1,000,000 against 30U/ml of MuIFN- $\gamma$  and contained 1.25 ng/ml endotoxin. IFN- $\gamma$  was Chinese hamster ovary (CHO) cell-derived recombinant MuIFN- $\gamma$  purified by affinity chromatography on anti-IFN- $\gamma$  mAb. The IFN- $\gamma$  was at an initial concentration of 300,000 IU/ml (endotoxin: 73pg/ml).

[0025] Animals were split into several groups as follows:

Group A: Animals were treated with a single intraperitoneal (IP) injection (100 $\mu$ l) of neat anti-IFN- $\gamma$  antibodies prior to wounding.

Group B: Animals were treated with a single intrader-

mal (ID) injection of 50 $\mu$ l or 25 $\mu$ l of anti-IFN- $\gamma$  antibodies (diluted with PBS) prior to wounding.

5 Group C: Animals were treated with a single ID injection of IFN- $\gamma$  (15,000 or 7,500 IU) prior to wounding.

10 Group D: Animals were treated with ID injections of IFN- $\gamma$  (15,000 or 7,500 IU) on day 0 prior to wounding and days 3 and 7 post-wounding.

15 Group E: Animals were treated with a single control IP injection of PBS (phosphate buffered saline) on day 0 prior to wounding (control).

Group F: Animals were treated with a single control ID injection of PBS on day 0 prior to wounding.

20 Group G: Animals were treated with an ID injection of PBS on day 0 prior to wounding and days 3 and 7 post-wounding.

25 [0026] Animals were killed by chloroform overdose on days 7, 14, 70 & 120 post-wounding. Wounds were excised and bisected for routine histology and immunocytochemistry. 7 $\mu$ m wax sections were cut and stained for Haemotoxylin and Eosin to assess cell invasion and re-epithelialisation, and for Masson's Trichrome to assess collagen deposition and orientation.

### Results

#### 35 Anti-IFN- $\gamma$ antibodies:

[0027] No difference was observed between control wounds and treated wounds at any time point in the animals treated with a single IP injection.

40 [0028] With a single ID injection of anti-IFN- $\gamma$ , there were no differences compared to controls at 7 and 14 days. However, by 70 and 120 days, marked differences in the orientation of the collagen fibres within the treated wound were observed.

45 [0029] Anti-IFN- $\gamma$  treatment is anti-scarring, improving the quality of dermal architecture, despite the prior art observations. While the fibres were still relatively small and compacted immediately under the epidermis, they are randomly orientated, whereas in the mid and deep dermis the collagen fibres were less compacted and were orientated in a "basketweave" fashion. Control wounds (scarred) had compacted parallel collagen fibres throughout the wound area.

#### 55 IFN- $\gamma$

[0030] At the early time points (7 and 14 days), all the IFN- $\gamma$ -treated wounds (in both injection regimes)

showed increased inflammation and angiogenesis in a dose-dependent manner, i.e. lower doses, although worse than control wounds, were not as bad as wounds treated with higher doses of IFN- $\gamma$ .

[0031] By 70 and 120 days, the wounds treated on days 0, 3 and 7 post-wounding with a high dose of IFN- $\gamma$  showed marked fibrosis (i.e. scarring). Macroscopically, the wounds were raised and, microscopically, densely packed collagen in large swirling bundles within the wound margins was observed. These treated wounds also showed residual inflammation at the base of the wound, compared to control wounds. Again, this scarring was dose-dependent, i.e. the greater the dose of IFN- $\gamma$ , the greater the scarring.

#### Discussion

[0032] Previous work has shown that administration of IFN- $\gamma$  to wounds inhibits collagen synthesis, suggesting that it may be useful as an anti-scarring agent. Other workers have shown that treatment of keloids or hypertrophic scars with IFN- $\gamma$  decreases the size of the scar.

[0033] Contrary to these findings, these experiments have shown that, very surprisingly, the early treatment of wounds with IFN- $\gamma$  causes fibrosis with raised scars that are packed full of collagen, whereas treatment of incisional wounds with antibodies to IFN- $\gamma$  results in improved healing with collagen fibres orientated in a "basketweave" fashion resembling normal dermis (i.e. scarring is reduced).

#### Claims

1. The use of an inhibitor of IFN- $\gamma$  in the manufacture of a medicament for promoting the healing of wounds or fibrotic disorders with reduced scarring.
2. The use of an inhibitor of IFN- $\gamma$  according to claim 1, the inhibitor comprising a neutralising antibody.
3. The use of an inhibitor of IFN- $\gamma$  according to either one of claims 1 or 2, the inhibitor being selected from any one of the group of a monoclonal antibody, a polyclonal antibody, a phage-derived antibody, a genetically engineered antibody and an antibody derived from a transgenic mouse.
4. The use of an inhibitor of IFN- $\gamma$  according to any one of claims 1-3 wherein the inhibitor prevents IFN- $\gamma$  interacting with its receptor.
5. The use of an inhibitor of IFN- $\gamma$  according to any one of the preceding claims for use in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.
6. The use of an inhibitor of IFN- $\gamma$  according to any one

of the preceding claims for use in conjunction with a composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

7. The use of an inhibitor of IFN- $\gamma$  according to any one of the preceding claim for use in conjunction with a composition for promoting the healing of chronic wounds.
8. The use according to any one of claims 1 to 7, comprising administering to a site of wounding or fibrosis an inhibitor of IFN- $\gamma$ .
9. The use according to any one of claims 1 to 8, comprising inhibiting between about 300 and about 30,000 IU IFN- $\gamma$ .
10. The use according to any one of claims 1 to 9 in which IFN- $\gamma$  is inhibited either immediately prior to wounding/onset or immediately after wounding/onset.

#### Patentansprüche

1. Verwendung eines Inhibitors für IFN- $\gamma$  bei der Herstellung eines Medikaments zur Förderung der Heilung von Wunden oder Fibroseerkrankungen mit verminderter Narbenbildung.
2. Verwendung eines Inhibitors für IFN- $\gamma$  gemäß Anspruch 1, wobei der Inhibitor einen neutralisierenden Antikörper umfaßt.
3. Verwendung eines Inhibitors für IFN- $\gamma$  gemäß einem der Ansprüche 1 oder 2, wobei der Inhibitor aus einem von der Gruppe eines monoklonalen Antikörpers, eines polyklonalen Antikörpers, eines phagenabgeleiteten Antikörpers, eines genetisch manipulierten Antikörpers und eines von einer transgenen Maus abgeleiteten Antikörpers ausgewählt ist.
4. Verwendung eines Inhibitors für IFN- $\gamma$  gemäß einem der Ansprüche 1-3, wobei der Inhibitor die Wechselwirkung von IFN- $\gamma$  mit seinem Rezeptor verhindert.
5. Verwendung eines Inhibitors für IFN- $\gamma$  gemäß einem der vorhergehenden Ansprüche zur Verwendung in Verbindung mit einem pharmazeutisch verträglichen Träger, Verdünnungsmittel oder Vehikel.
6. Verwendung eines Inhibitors für IFN- $\gamma$  gemäß einem der vorhergehenden Ansprüche zur Verwendung in Verbindung mit einer Zusammensetzung zur Förderung der Heilung von Wunden oder Fibroseerkrankungen mit verminderter Narbenbildung.

7. Utilisation d'un inhibiteur de l'IFN- $\gamma$  conformément à l'un des revendications 1 à 6, comprenant l'administration à un site de blessure ou de fibrose d'un inhibiteur de l'IFN- $\gamma$ .
8. Utilisation conformément à l'un des revendications 1 à 6, comprenant l'administration d'un inhibiteur de l'IFN- $\gamma$  à un site de blessure ou de fibrose.
9. Utilisation conformément à l'un des revendications 1 à 6, comprenant l'administration d'un inhibiteur de l'IFN- $\gamma$  à un site de blessure ou de fibrose.
10. Utilisation conformément à l'un des revendications 1 à 6, comprenant l'administration d'un inhibiteur de l'IFN- $\gamma$  à un site de blessure ou de fibrose.

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#### Revendications

1. Utilisation d'un inhibiteur de l'IFN- $\gamma$  dans la fabrication d'un médicament pour activer la cicatrisation de blessures ou de lésions fibreuses avec des cicatrices moins marquées.
2. Utilisation d'un inhibiteur de l'IFN- $\gamma$  selon la revendication 1, l'inhibiteur comprenant un anticorps neutralisant.
3. Utilisation d'un inhibiteur de l'IFN- $\gamma$  selon l'une quelconque des revendications 1 ou 2, l'inhibiteur étant choisi parmi l'un quelconque dans le groupe d'un anticorps monoclonal, d'un anticorps polyclonal, d'un anticorps dérivé d'un phage, d'un anticorps modifié génétiquement et d'un anticorps provenant d'une souris transgénique.
4. Utilisation d'un inhibiteur de l'IFN- $\gamma$  selon l'une quelconque des revendications 1 à 3, dans laquelle l'inhibiteur empêche l'IFN- $\gamma$  d'interagir avec son récepteur.
5. Utilisation d'un inhibiteur de l'IFN- $\gamma$  selon l'une quelconque des revendications précédentes, à utiliser conjointement avec un véhicule, diluant ou excipient pharmaceutiquement acceptable.
6. Utilisation d'un inhibiteur de l'IFN- $\gamma$  selon l'une quelconque des revendications précédentes, à utiliser conjointement avec une composition pour activer la cicatrisation de blessures ou de lésions fibreuses avec des cicatrices moins marquées.
7. Utilisation d'un inhibiteur de l'IFN- $\gamma$  selon l'une quelconque des revendications précédentes, à utiliser

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conjointement avec une composition pour activer la cicatrisation de blessures chroniques.

8. Utilisation selon l'une quelconque des revendications 1 à 7, comprenant l'administration à un site de blessure ou de fibrose d'un inhibiteur de l'IFN- $\gamma$ .

9. Utilisation selon l'une quelconque des revendications 1 à 8, comprenant l'inhibition entre environ 300 et environ 30 000 UI d'IFN- $\gamma$ .

10. Utilisation selon l'une quelconque des revendications 1 à 9, dans laquelle l'IFN- $\gamma$  est inhibé soit immédiatement avant une blessure/début d'une lésion fibreuse soit immédiatement après une blessure/début de lésion fibreuse.